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Velocity Selective Recording: A Demonstration of Effectiveness on the Vagus Nerve in Pig

Benjamin Metcalfe¹, Thomas Nielsen² and John Taylor¹

Abstract—Neural interfaces that can both stimulate and record from the peripheral nervous system are an important component of future bioelectronic devices. However, despite a long history of neurostimulation, there has been relatively little success in the design of a chronically implantable device for recording from peripheral nerves. This fundamental road block must be overcome if the design of advanced implantable devices is to continue. In this paper, we demonstrate the effectiveness of one method: velocity selective recording, a method that has been proposed as a tool for online neural recording that does not require training. We present results and analysis from in-vivo recordings made on the right vagus nerve of pig using a multiple-electrode cuff as a chronically implantable recording array.

I. INTRODUCTION

Neural interfaces are a highly topical research area, important for not only fundamental science and electrophysiology, but also for the development of medical devices and neural prostheses. Interfaces that can both record from and stimulate the nervous system are of particular interest; however, the challenges faced with neural recording have limited the development of such devices. Multi-unit recordings from the brain are common, whereby action potentials are frequently differentiated based on changes in shape that arise from spatial distributions of the recording electrodes. Recording from the peripheral nervous system has been less widely adopted, perhaps because of the relative size and fragility of small peripheral nerves. There are also significant issues with very low signal amplitudes and a lack of differentiation based on waveform shape. Current approaches typically include the use of spatiotemporal filters, matched filtering, tomography, or discrimination based on conduction velocity [1]. Most techniques make use of multiple-electrode structures, normally with electrodes distributed axially, or in the case of velocity discrimination, distributed longitudinally.

The concept of velocity discrimination of action potentials has a long history; however, it is only recently that the first recordings of action potentials from the peripheral nervous system have been made using this method. Initially using electrical stimulation to recruit large-amplitude evoked Compound Action Potentials (eCAPs), in worm [2], frog [3], [4], pig [5] and rat [6]. The first successful attempt at recording physiological (i.e. naturally occurring) action potentials using this method was made by Metcalfe *et al.*

using hook electrodes in rat [6]. This work was further developed by recording respiration afferents from a much larger nerve (the right vagus) in pig, using a chronically implantable cuff electrode [7]. In this paper, we present an analysis of the electrically evoked potentials within pig vagus, including a discussion on the validation of previous approaches, and evaluate the effectiveness of velocity discrimination as a tool for online neural recording and analysis.

II. MATERIALS AND METHODS

A. Electrode Design

Two different cuffs were manufactured and implanted on the intact right vagus nerve of an adult pig, a tripolar stimulation cuff and a multiple-electrode recording cuff, both were manufactured according to the technique described by Haugland [8]. The tripolar stimulation cuff was approximately 12 mm long, with an inner diameter of 2.4 mm, containing three 1 mm wide platinum-iridium ring electrodes with approximately 5 mm centre-to-centre pitch. The multipole recording cuff was approximately 42 mm long, with an inner diameter of 2.4 mm, and contained ten 1 mm wide platinum-iridium ring electrodes with 3.5 mm centre-to-centre pitch.

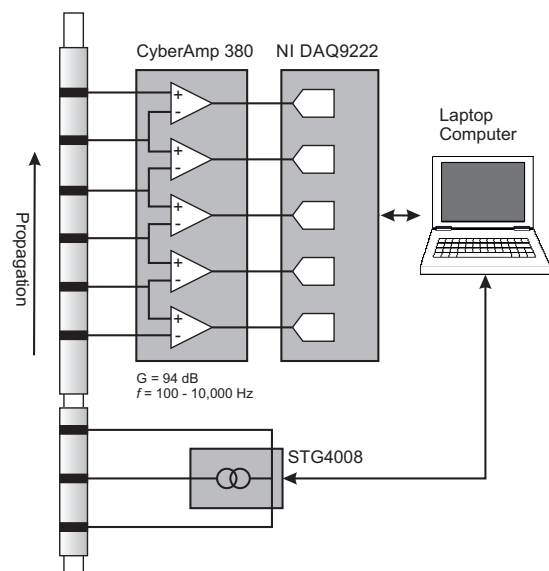


Fig. 1. Schematic representation of the electronic apparatus showing the tripolar stimulation cuff, the multiple-electrode recording cuff and associated equipment. The illustrated electrode count has been reduced for simplicity.

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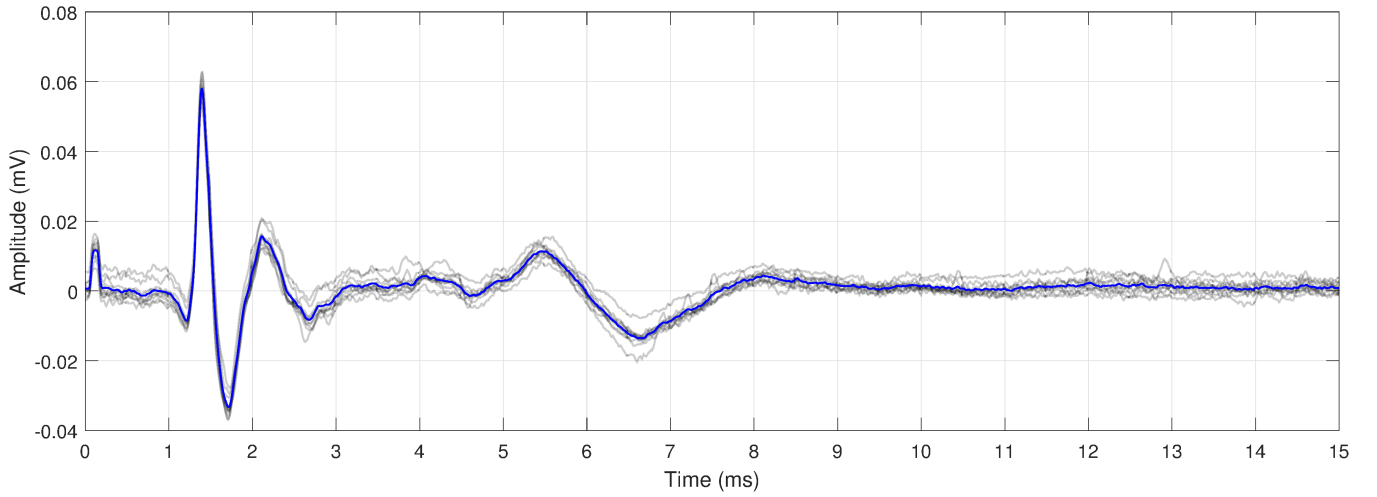


Fig. 2. Time domain recording of a single channel from the maximal stimulation event of 5 mA. Each thin line represents the trace from each of the ten repeats and the thick line is the mean average waveform over all repeats. Clearly visible are the stimulation artefact (0.2 ms), the fast eCAP (1.2 ms) and the slow eCAP (5.5 ms).

B. Surgical Methods

The experimental procedures involving animal models described in this paper were approved by the *Danish Animal Experiments Inspectorate* (approval no. 2013-15-2934-00753), as well as the care and use of laboratory animals as described by the *U.S. National Institutes of Health*. An adult female Danish Landrace pig of approximately 50 kg was sedated with zoletil administered by the intramuscular route, transported to the hospital, and then intubated and anaesthetized with sevoflurane and zoletil. The animal was mechanically ventilated at 15 cycles per minute.

An incision of approximately 20 cm was made on the right side of the trachea to expose the right cervical vagus nerve, and a section of approximately 15 cm of the nerve was freed from surrounding tissues. The recording cuff was placed around the nerve at the caudal end of the freed section; the stimulation cuff was placed around the nerve at the cranial end. A silicone sheet was placed around the cuff to minimise current leakage, and the cuff was closed with ligatures at each end and in the centre. The animal was grounded electrically via a subcutaneous stainless-steel probe inserted at the abdomen.

After all surgical procedures had been completed, the anaesthesia was switched to a mixture of ketamine, midazolam, and fentanyl (administered intravenously) and the animal was left to stabilise for at least 20 minutes before performing the experiments. The animal was euthanised after completion of the experiments by an overdose of pentobarbitone.

C. Electronic Apparatus

The stimulation apparatus consisted of a programmable stimulator (STG4008 - Multichannel Systems) that was configured to produce charge-balanced square pulses. Each pulse comprised a primary cathodic phase of 100 μ s duration and amplitude ranging from 0 - 5 mA in steps of 100 μ A, followed, after a delay of 100 μ s, by an anodic phase of 2 ms

duration and 5% of the cathodic amplitude. This corresponds to a peak charge density of 6.6 μ C/cm². The delay between each stimulation event was 200 ms and the entire stimulation profile was repeated ten times. The range of stimulation currents was designed to recruit selectively (i.e. stimulate the production of action potentials), the whole range of axons within the nerve. The larger diameter axons, which produce action potentials with high conduction velocities, have a lower stimulation threshold than the smaller diameter axons. As the stimulation current is increased, a greater proportion of the total axons within the nerve will be recruited to produce action potentials. It is assumed that all the axons will produce their action potentials concurrently with the stimulation event, and thus that action potentials from similar axons will propagate synchronously along the nerve. These action potentials, when recorded by the cuff, will sum to produce the large amplitude eCAP.

The recording apparatus consisted of a CyberAmp 380 with AI402 SmartProbes in a bipolar configuration. The overall amplifier voltage gain was 94 dB and each channel was filtered with a passband extending from 100 Hz to 10 kHz. In contrast to hook electrodes, the expected amplitudes of the extracellular potentials recorded with a cuff are substantially lower (typically, using cuffs, 1 - 10 μ V for physiological ENG and 10 - 100 μ V for CAPs). The amplified and filtered signal was digitised using a bank of high-speed analogue to digital converters (NI9222 - National Instruments) providing simultaneous sampling of all nine bipolar recordings at a sample rate of 100 kS/s with 16-bit resolution. A schematic representation of the electronic apparatus and the cuff electrodes in-situ on the vagus nerve is shown in Fig. 1.

D. Data Analysis

After digitisation, all recordings were analysed using a process known as *delay-and-add* beamforming. Each bipolar recording V_{Bi} is delayed relative to the end recording by an

interval that depends on both the electrode spacing and the conduction velocity of the action potential. If the delay inserted between the first two channels (V_{B1}, V_{B2}) is dt then the delay inserted between the first and third channels (V_{B1}, V_{B3}) is $2dt$, and so on. After insertion of the delay, the channels are summed together; clearly the result of this summation will be largest if the artificial delay cancels out the naturally occurring delay caused by the finite conduction velocity of the action potential. The inserted delay can be swept over a range of values to identify any peaks that correspond to correlated action potentials. There are multiple advantages of this process. Firstly, if the noise sources are uncorrelated (a reasonable assumption), then there is an increase in effective SNR of approximately \sqrt{C} , where C is the number of channels [9]. Secondly, any contaminating signals that appear as common mode are automatically rejected. Finally, it is possible to differentiate *afferent* from *efferent* activity by using positive or negative values of delay.

III. RESULTS

A. Electrically Evoked Compound Action Potentials

A continuous recording was made throughout all 512 stimulation events, the recording apparatus simultaneously sampling nine bipolar channels within the recording cuff. Fig. 2 is a time domain recording of a single bipolar channel (the most proximal to the stimulation cuff) corresponding to a stimulation of 5 mA. The thin lines represent the traces from each of the ten repeats, and the thick line is the mean average waveform. Clearly visible is a stimulation artefact (0.2 ms), a fast eCAP (1.2 ms) and a slow eCAP (5.5 ms). The stimulation artefact was present in all channels within the recording cuff, appearing concurrently with the onset of stimulation. The mean amplitude of the fast eCAP is $57.8 \mu\text{V}$ and of the slow eCAP $11.3 \mu\text{V}$.

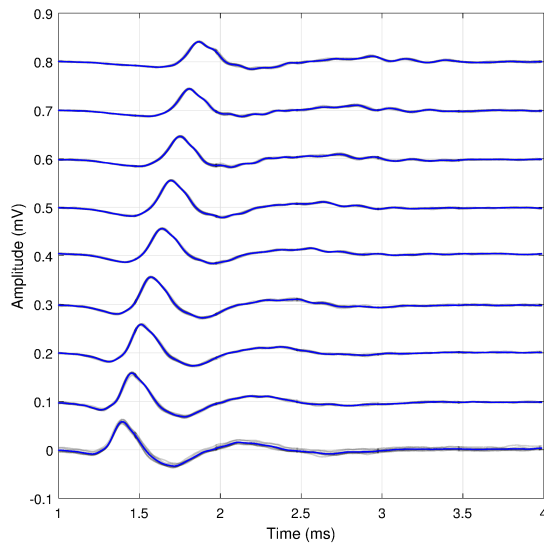


Fig. 3. Time domain recordings from all nine channels within the recording cuff for a stimulation level of 5 mA, zoomed to show only the fast eCAP. The propagation delay is evident as the eCAP travels along the cuff, the ordinate offsets have been added for presentation purposes.

Fig. 3 shows the fast eCAP propagating along the full length of the cuff; each trace is a successive bipolar recording from adjacent pairs of electrodes, and the time delay apparent between the channels is a result of the conduction velocity of the eCAP. The morphology of the eCAP showed some variation over the length of the recording cuff, the amplitude generally reducing, and the width increasing. The traces in Fig. 3 are overlays from each of the ten repeats and demonstrate that the change in morphology was consistent over the experiments. A similar effect was noted for the slower eCAP.

The fast eCAP was first observed at a stimulation current of approximately 0.25 mA, as the current was increased the amplitude of the eCAP also increased - indicative of increasing recruitment. At approximately 2.5 mA, the slower eCAP became visible, and its amplitude increased with increasing stimulation current. Fig. 4 illustrates the absolute and the average amplitudes of the two eCAPs over the entire range of stimulation currents.

B. Conduction Velocities

To measure the conduction velocity of each eCAP, the nine channels of data were beamformed using the delay-and-add process. A time window of 15 ms was extracted from the recordings of all nine channels for two different stimulation levels (0.5 mA and 5 mA). The range of velocities considered was 5 - 100 m/s, with an increment of 0.5 m/s. After beamforming, the maximum value was taken over the time window for each velocity, producing the *intrinsic velocity spectra* (IVS) shown in Fig. 5 [6]. At a stimulation level of 5 mA there are two clearly visible peaks that correspond to the fast eCAP ($v = 58 \text{ m/s}$) and the slow eCAP ($v = 15 \text{ m/s}$) respectively. The 0.5 mA stimulation level only recruits the fast eCAP ($v = 58 \text{ m/s}$) albeit with a reduced

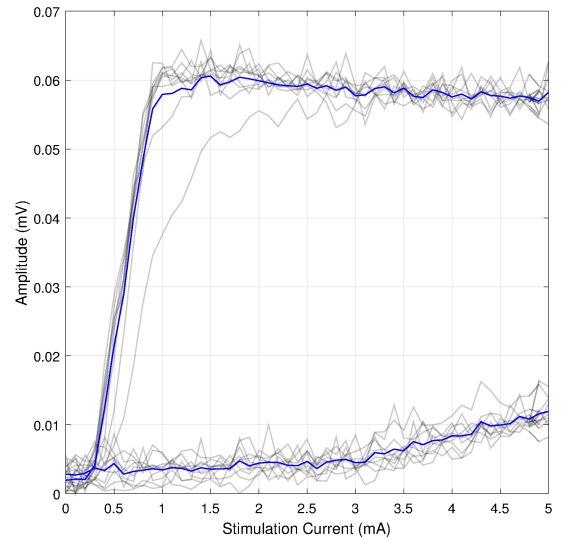


Fig. 4. The peak amplitude of the fast and slow eCAP for each stimulation level. The fast eCAP is first recruited at 0.25 mA, and the slow eCAP at approximately 2.5 mA. The thin lines show each of the ten repeats and the thick line the mean average of all repeats.

overall amplitude, indicative of only partial recruitment of these axons. The thin lines represent the IVS for each of the ten repeats and the thick line the mean average.

IV. DISCUSSION

Two dominant eCAPs were observed, these likely are $A\delta$ (58 m/s) and $A\beta$ (15 m/s) axons; probably baroreceptors in the lungs and heart, parasympathetic efferent fibres innervating the heart, and possibly laryngeal motor fibres. The vagus nerve is expected to also contain many C fibres, which are unmyelinated and with conduction velocities below 5 m/s. The square law relationship between fibre diameter and recorded amplitude is probably why no C fibre activity is observed in these recordings [10]. The amplitude of the 58 m/s eCAP decreased over the length of the recording array in all the experiments and also increased in width. This effect has been observed in other studies of the eCAP recorded from pig median nerve [5]. Possible explanations for this effect include variations in electrode-tissue impedance, or modulation of the eCAP via an unknown mechanism; neither of these theories fully explain the observed characteristics. Instead, we propose that the underlying axonal morphometry of large nerves may be multi-modal (that is to say that a histogram of axon diameters has multiple modes).

The change in eCAP morphology over the length of the recording array may be explained by a broad mode centred on 58 m/s. As the axons with similar, but not identical, diameters produce action potentials that diverge in time. Further, there appear to be only two eCAPs for a large nerve that should support many fibre types. A multimodal distribution would likely produce as many large eCAPs as there were modes, and these may have larger amplitudes than eCAPs from axons that are not near the modes.

A common technique in this type of recording is the application of bandpass filters that act to reduce the width

of the eCAP in the time domain, and in doing so increase the apparent selectivity in the velocity domain [3]. This technique is valid for individual action potentials; however, we do not believe it is valid when applied to eCAPs that are superpositions of many *similar* action potentials. Fundamentally, the limit of discrimination is a result of the underlying fibre distributions and the length of the recording array. As the separation between the stimulation and recording sites increases, the individual action potentials that form the eCAP will spread out in space and time; although this would enable a finer level of discrimination it would also reduce the overall SNR. The limit of this case might be considered the physiological neural activity, which we have discussed previously [7].

V. CONCLUSION

In this paper, we have shown a demonstration of velocity selective recording to the analysis of the electrically evoked compound action potentials recorded in-vivo from the right vagus nerve of pig. The technique requires no training and very limited processing. The recordings suggest two main groups of axons within the nerve, and this could be confirmed with the aid of histological studies to produce axonal diameter histograms.

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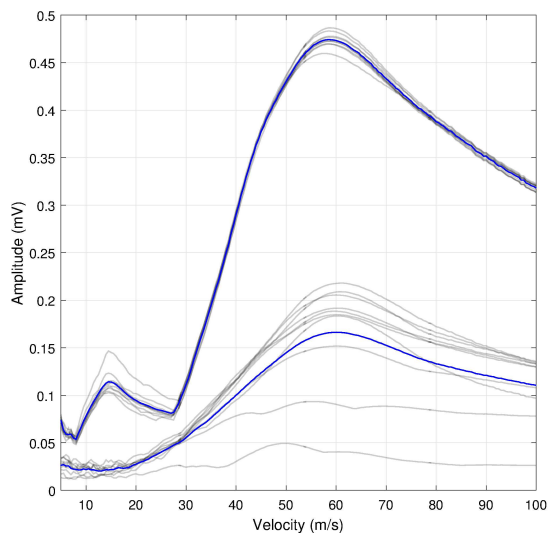


Fig. 5. The IVS for stimulation currents of 0.5 mA and 5 mA. Clearly visible are two populations corresponding to the fast eCAP ($v = 58$ m/s) and the slow eCAP ($v = 15$ m/s). The thin lines represent the IVS for each of the ten repeats and the thick line the mean average of all repeats.